Biology and Physiology of Targeted Radionuclide Therapy (TRT)

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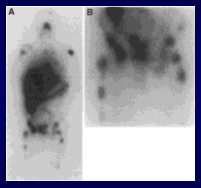
Outline: Biology/Physiology of Tumour Targeting. An Engineering Talk

1. Steps on the way to a tumour
2. Types of labeling
3. Uptake measurements
4. Classes of targeting agents (will discuss 5)
5. Comparing agents using figures of merit
6. Comparing using impulse response functions
7. Promising strategies (blocking, stealth, and multi-step targeting)

Motivation for Targeted Radionuclide Therapy(TRT)

1. Too many disease locations for surgery or external beam therapy
2. Prior failure of chemotherapies
3. Possible undetected sites of primary and/or metastatic disease

The Clinical Situation



Medullary thyroid cancer patient at 48 h post anti-CEA intact antibody injection. In-111 is the radiolabel.

Probable Steps in Tumour Targeting

1.Blood injection (iv) to access all possible sites (is agent stable?) Note that direct injection is used for sphere treatment of limited hepatic disease

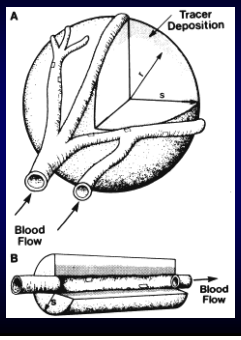
2. Extravasation into tumour space due to fenestrations (is agent small enough to fit thru gaps?)

3. Diffusion down a concentration gradient into the intra-tumour space (is there retrograde convection?)

4. Fixing to the tumour cell (or at least close enough for radiation therapy work)

5. Possible movement to inside the tumour cells (internalization)

Blood Flow to the Tumour



Nanoscale Engineering

Two items are needed to build a radiopharmaceutical(RP):

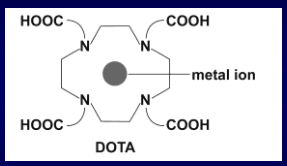
1. A physical/chemical entity which targets tumour cells and/or their associated molecules. This is the pharmaceutical.
2. A radiolabel attached to the pharmaceutical so that imaging and therapy are possible. Adding this aspect generates the radiopharmaceutical. Note that non-radioactive therapy is also possible with some RP agents.

Methods and Limitations of Radiolabeling

Iodine labeling is one-step. Smaller amounts (100 µg) of pharmaceutical required. Several isotopes such as ^{123}I, ^{131}I and ^{124}I. Dehalogenation is an issue *in-vivo* due to thyroid competition for iodine.

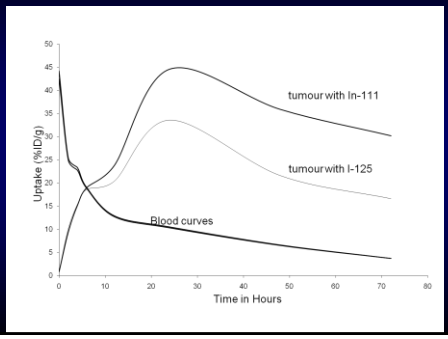
Radiometal labeling requires a bifunctional chelating agent. Thus, two chemical steps and larger amounts of pharmaceutical (500 µg) are needed. Yet many radiometals in the periodic table. Can adjust gamma, beta energies and lifetimes. Examples are ^{111}In, ^{90}Y, ^{177}Lu, ^{64}Cu.

The most common chelating agent for radiometals is DOTA



Notice the four COOH arms that envelope the metal ion. Metal binding is a quantum- mechanical process.

Murine Biodistribution of scFv-FcA antibody with twin labels



Uptake Concept; Proof of Targeting

Uptake is percent injected dose per gram of sampled tissue. If we assumed a uniform biodistribution of the agent in a mouse, then we would have an organ or tumour uptake (u) given by:

100% ID/25 gm = 4% ID/gm

Where ID is injected dose (MBq) corrected for radio decay.

If this value is exceeded in a murine tumour, then we have evidence of specific targeting. Absorbed dose rate is proportional to uncorrected uptake so that therapy applications are clear.

Two Formats for Biodistribution Data

We generally wish to show the organ uptake corrected for radio decay (% ID/gm). Samples are obtained by dissection, or quantitative imaging (PET and SPECT) and counted. Results are termed the (pharmacological) biodistribution and are a standard result given in a journal article or patent application. Label may have been removed, however.

Alternatively, we may show radioactivity present in the organs in which case there is no correction for decay and one shows the % IA/gm where IA refers to injected activity. This table is important to the physicist doing dose estimations. Thus, both formats are needed.

Some Classes of Engineered Agents for TRT (not a complete list)

1. Liposomes
2. Antibodies and all their kith and kin
3. Small proteins (hormone-like)
4. Short (20 to 30 base pairs) pieces of DNA or RNA; aptamers.
5. Nanoparticles

"Stone-Age" Agent for TRT: The Liposome

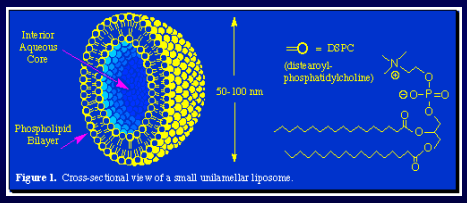


Figure 1. Cross-sectional view of a small unilamellar liposome.

Adjustable Parameters: Size, charge, chemical makeup, stability, and surface adjuncts. Image courtesy of F. Hawthorne

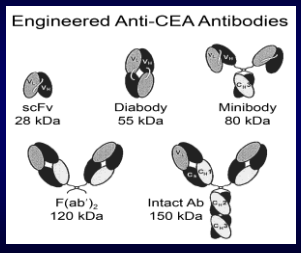
Liposome Biodistribution Results

Advantages: Variable electric charge, size, and non-specificity

Disadvantages: Instability in blood, non-specificity, RES uptake

organ	Uptake (%ID/g) At 24 h	Uptake with block At 24 h
Liver	14.6 +/- 0.8	14.0 +/- 2.1
Spleen	18.1 +/- 1.6	13.7 +/- 1.6
Blood	6.6 +/- 0.8	6.8 +/- 1.5
EMT6 tumour	18.5 +/- 2.4	28.7 +/- 2.6

The Second Type of Targeting Agent: The Antibody



Parameters: MW (size), valence, chemical makeup(mouse and man)

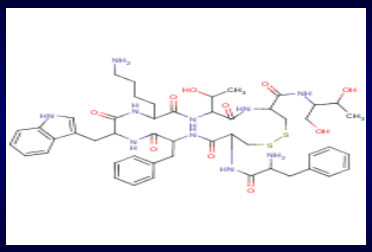
Antibody as a TRT Agent

Advantages: Specific, variable MW, may elicit patient's immune response, can be combined with other proteins (e.g., bi-specific antibody), other types of agent (liposome), or to carry a chemotherapy molecule to the tumour.

Disadvantages: Patient immune response (HAMA, HACA, HAHA) after repeated applications. HAMA is human anti-mouse antibody.

Organ	Uptake (%ID/g) at t = 0 h	Uptake at t = 48 h
Liver	7.9 +/- 1.0	2.7 +/- 0.2
spleen	3.5 +/- 0.5	1.9 +/- 0.2
blood	38.3 +/- 4.3	13.2 +/- 2.0
LS174T Tumour	0.9 +/- 0.1	42.6 +/0 9.1

The Third Type of Targeting Agent: the Small Protein (Octreotide)



Parameters: No of amino acids, target molecule

Small Protein (Octreotide) as a TRT Agent

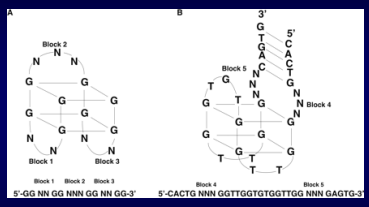
Advantages: Simple to construct, no immune response

Disadvantage: High renal %ID/g, reduced tumor uptake

Organ	Uptake (24 h with ^{111}In)
Kidneys	30 %ID/g
Liver	0.1
Carcinoid Tumor	0.25

Bernhardt et al JNMB 30, 253, 2003

Fourth Type of TRT Agent: The Aptamer



Parameters: No of base pairs, target molecule,

Aptamer as a TRT Agent

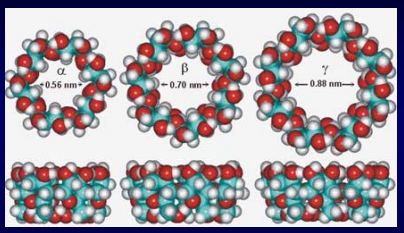
Advantages: Specific to target, easily made (1 month), no immune response, rapid blood clearance.

Disadvantages: Rapid blood clearance and low tumor uptake.

Organ	Uptake with ^{99m}Tc at 10min
Liver	32 %ID/g
Spleen	12
Tumour (glioblastoma)	4.1

Hicke et al JNM 47, 668, 2006. Aptamer against tenascin-C

A Fifth Type of RP Agent, Nanoparticle



Cyclodextrin developed by Mark Davis of Caltech. Description appeared on PBS; program seen in Canada.

Class 5: Nanoparticle as a TRT Agent

Advantages: Variable size, content, entirely man-made, does not need radionuclide(!) for therapy, can enter cell, rapid clearance

Disadvantages: Small size → low tumour uptake, kidney, bladder and liver accumulation. Rapid clearance, stability may be questioned

Organ	Uptake (40 min) in Mouse ; siRNA is content. ^{64}Cu label
Liver	25 %ID/g
Kidney	10
Bladder	60
Neuro2A Tumour	1

Bartlett et al PNAS 104, 15549, 2007.

The "Problem of Plenty"

1. There are multiple classes (types) of potential tumour targeting agents. These types are not mutually exclusive.

2. Each type may have an almost astronomical number of variants such as 20^{1500} for an intact antibody.

3. Yet the number of patients for a clinical trial is limited; approximately 3% of available patients will volunteer for a cancer protocol.

Thus, some sort of pre-clinical selection process is needed.
I propose a figure of merit (FOM) to separate one agent from another.

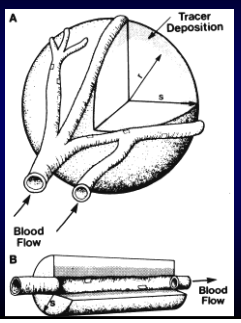
Evidence for tumor uptake to be a function of tumour mass

From both animal and clinical data sets, there is evidence that many tumours have uptake (u_T) that is a function of the tumour mass (m). Generally, the larger the tumour, the smaller the uptake. We expect that the relationship is similar to:

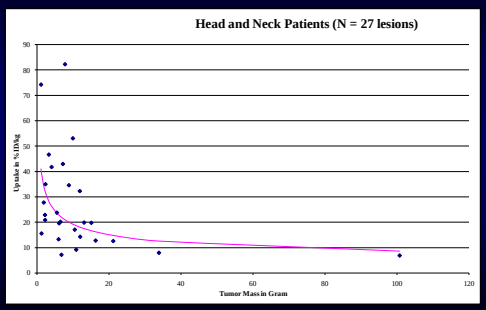
$$u_T = u_0 / m^b$$

Where $0.33 < b < 0.60$

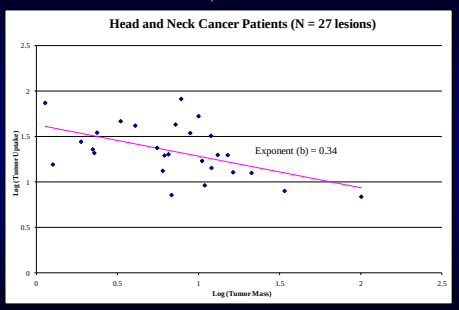
Schematic of Blood Flow to the Tumour



Head and Neck Cancer; de Bree et al EJNM 25:1998.



Head and Neck Cancer; de Bree et al EJNM 25: 1998.



Figures of Merit for Radiopharmaceutical(RP) Comparison

For RPs, the historical imaging figure of merit is the ratio R of tumour uptake to blood uptake:

$$R = u_T/u_B.$$

Yet this neglects the amount of RP in the tumour so that it is only a necessary, not a sufficient index. I have defined another Imaging Figure of Merit (IFOM) based on the statistical likelihood of imaging in a finite time. One may then compare these two indicators for a set of 5 cognate proteins developed at City of Hope against CEA.

$$\text{IFOM} = \frac{1}{\Delta t} = \frac{[\,CV(x)]^2\,\varepsilon\,V\,[1-1/R]^2\,u_T(t)\,\exp(-\lambda t)}{[1+1/R]}$$

Justification of the use of IFOM instead of the Traditional Ratio (R) of tumour/blood uptake

1. Selection of Optimal Imaging Time for a given agent:

IFOM predicts a finite optimal time; R is monotone increasing such that an optimal time cannot be determined for 5 cT84.66 cognates.

2. Size of lesion for best imaging:

IFOM predicts a $m^{2/3}$ dependence ; R goes approximately as $m^{-1/3}$ such that R is optimal for the smallest lesions. This is absurd.

3. Radiolabel:

IFOM goes as exp(-λt) ; R is independent of label so all radionuclides are said to be equal for imaging. This is equally absurd.

Figures of Merit based on Biodistribution may not be Enough

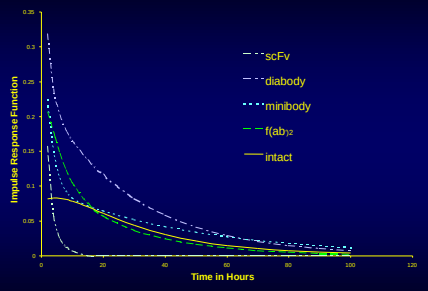
It may be that the agent is “intercepted” by normal organs on its way to the tumour. In this case, the uptake of the lesion is reduced so that biodistribution values may not indicate the actual effectiveness of the tracer in the lesion. What else can we look at?

Deconvolution: Which Antibody Agent has the most Potential for Future Engineering?

$$u_{Tumour}(t) = \int u_{Blood}(t-\tau)\; h(\tau)\, d\tau$$

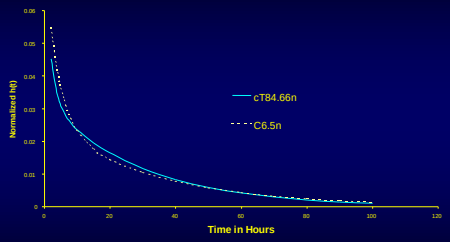
h(τ) is the Impulse Response Function and is the unknown.
Other functions in the analysis are given by the biodistribution data.

Impulse Response Functions h(τ) for Colon Tumour



The diabody has the optimal h(τ) function among 5 α-CEA cognates

Normalized Diabody Impulse Response Functions



It appears that normalized h(t) functions are similar. Are there generic normalized impulse response functions?

Additional Strategies involved in TRT:

1. Stealth Technology to Increase circulation times and ↓ immune response in patient. PEG.

2. Multi-step targeting; e.g. cold antibody followed by small, labeled ligand with rapid sequestration by the tumour.

3. Blocking Step. Use cold material to saturate normal organ(s). Not clearly understood, but seems to be important.

4. Multi-modality therapy with chemo, radiation sensitizers, external beam, hyperthermia, siRNA.

Current Situation: TRT and Various Agents

Tumour uptakes can attain ≈100%ID/g in mouse or ≈ 50%ID/kg in patients. Higher MW → higher uptake, but slower clearance. Thus, larger marrow absorbed dose.

Only Lymphoma (B-cell) is FDA-approved for TRT treatment. Lymphoma and solid tumour absorbed doses are comparable ≈ 20 Gy at MTD. Red marrow dose limits the procedure.

Conclusions Regarding Targeting Agents

Multiple classes of agents and variable labels make choices for imaging and therapy difficult. Because of immune response, smaller MW agents are currently being favored over larger entities.

Strategies are being developed to enhance uptake using blocking, stealth, multi-step and other techniques. Largely a "black magic" arena with little numerical understanding.

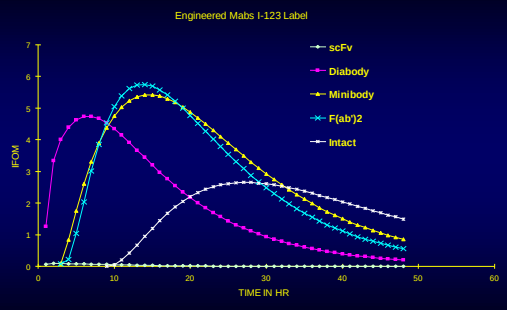
While now a standard B-cell lymphoma therapy, the method may be expanded to include non-radioactive agents to stop tumour growth. Requires large amounts of material.

Thank you for your attention !

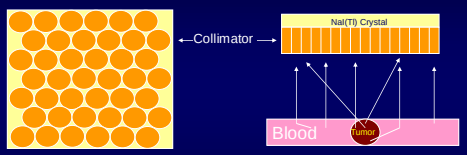
Questions: Please use the email address below for contact:

lwilliams@coh.org

IFOM for the set of 5 cT84.66 cognates



IFOM Verification with Monte Carlo (MCNP) Simulation of NM camera

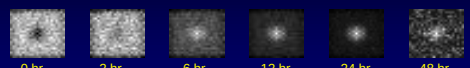


- Collimator hole: 0.25cm diameter
- Septal thickness (lead): 0.03cm
- Collimator thickness: 2.5cm
- NaI Crystal thickness: 3/8 inch

- LEHR collimator
- 20 mins scan of I-123 (20 CPU hrs)
- 20% energy window
- Radioactivity concentration data from animal biodistribution

IFOM Verification with Monte Carlo Simulated NM images (20 min scan)

I-123 Labeled GS Flex Minibody



	0 hr	2 hr	6 hr	12 hr	24 hr	48 hr
IFOM	0	0.05	2.6	5.23	4.1	0.85
R	0.11	0.70	2.25	4.19	8.14	22.69

I-123 Labeled Diabody

	0 hr	2 hr	6 hr	12 hr	24 hr	48 hr
IFOM	0	3.34	4.74	3.91	1.56	0.2
R	0.04	2.87	6.75	23.3	275	38649

Additional Strategies 2: Multi-Step Targeting of Tumors

To enhance uptake and control absorbed doses more directly, a 2- or 3-step procedure is done instead of the single step. Invented by Mears and Goodwin, the process is as follows:

1. Injection of an unlabeled agent against antigen “x” but thereby exposes a new antigen “y” on its surface.
2. Subsequent injection of a labeled agent targeted to “y”.
This material usually has a very low MW and clears rapidly.
2A. A clearing step, between 1 and 2, may also be used to remove unlabeled material and reduce the likelihood of complexes in the blood.

Additional Strategies 3: Adding a Blocking Step before the Injection of a labeled agent to enhance tumor uptake

We may wish to saturate (“block”) some biological sequestration process.

1. Blocking of the RES using unlabeled liposomes for subsequent Injection of labeled liposomes. Shown in earlier table.

2. Injection of cold anti-CD20 antibody to saturate receptors on normal B cells with a subsequent injection of 90Y-labeled anti-CD20 antibody. Presently done in both Bexxar and Zevalin clinical studies

Additional Strategies 4: Multiple Modality Therapy

1. External beam therapy prior to TRT
2. Hyperthermia with TRT
3. Radiation Enhancers given prior to or simultaneous with TRT
4. Chemotherapy with TRT

Comparison of Intact cT84.66 Mab in Nude Mice and Colorectal Cancer Patients

PK Parameter	Murine Result (^{131}I)	Clinical Result(^{111}In)
k_{12}	1.4 +/- 3 X 10^{-3} h^{-1}	17 +/- 7X 10^{-3} h^{-1}
k_{21}	37 +/- 61 X 10^{-3} h^{-1}	13 +/- 3 X 10^{-3} h^{-1}
K_{13}	87 +/- 21 X 10^{-3} h^{-1}	7 +/- X 10^{-3} h^{-1}
K_{31}	0.60 +/- 0.15 X 10^{-3} h^{-1}	28 +/- 12 X 10^{-3} h^{-1}
K_{30}	12 +/- 14 X 10^{-3} h^{-1}	7 +/- 2 X 10^{-3} h^{-1}
K_{40}	0.15 +/- 0.75 h^{-1}	7 +/- 1 X 10^{-3} h^{-1}
V	2.3 +/- 0.20 ml	5.36 +/- 0.52 X10^{+3} ml
f_{bl}	0.12 +/- 0.01	0.17 +/- 0.01

Impulse Response Function Moments and Mean Residence Time (T_R) for the cT84.66 Cognate Family Labeled with Radioiodines

Antibody	h_0	h_1	T_R
ScFv	0.94	1.81 h	1.92 h
Diabody	6.06	101.5	16.8
Minibody	4.66	137.1	29.4
F(ab')$_2$	3.75	79.8	21.3
Intact cT84.66	3.33	103.2	31.0